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Oxygen Production and Consumption in the Sacoglossan (=Ascoglossan) *Elysia chlorotica* Gould

by

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Abstract. A naturally occurring population of *Elysia chlorotica* Gould (Opisthobranchia: Sacoglossa) composed of a mixture of individuals ranging from dark green to non-green in color was found in the Minas Basin, Nova Scotia, Canada. This species is usually dark green in color because of endosymbiotic chloroplasts derived from their food alga *Vaucheria* sp. Individuals from this population were examined for O_2 production and consumption. A correlation between chlorophyll content and O_2 production was found.

INTRODUCTION

SINCE THE FIRST identification of sacoglossan endosymbionts as chloroplasts (KAWAGUTI & YAMASU, 1965), many such associations have been described, especially in reference to plastid origin (TAYLOR, 1968; TRENCH *et al.*, 1969; GREENE, 1970a; TRENCH, 1975) and functional capacity, including carbon fixation (GREENE, 1970b; TRENCH, 1973; STIRTS & CLARK, 1980; CLARK *et al.*, 1981) and oxygen production (Brandt, 1883, *in* TAYLOR, 1968; KAWAGUTI & YAMASU, 1965; TRENCH *et al.*, 1969; TRENCH, 1975; GRAVES *et al.*, 1979). These authors have demonstrated a net O_2 production under illumination and attributed it to the presence of endosymbiotic chloroplasts.

Sacoglossans are herbivorous and feed by slitting or piercing a food plant with their radula and suctorially removing plant sap and chloroplasts (TRENCH *et al.*, 1969; JENSEN, 1983). The chloroplasts are phagocytized by the slug's digestive cells (MUSCATINE *et al.*, 1975; MCLEAN, 1976) in which they are maintained for variable periods of time depending upon the sacoglossan and algal species (TAYLOR, 1968; TRENCH, *et al.*, 1969; HINDE & SMITH, 1972; CLARK *et al.*, 1981).

The presence of chloroplasts in the digestive diverticula typically colors the slug identically to the plastid source, and comparison of pigment spectra of the slug and possible food choices is commonly used to determine the actual food plant (TAYLOR, 1968; TRENCH *et al.*, 1969; GREENE, 1970a; TRENCH, 1975). Chlorophyll content has been shown to be indicative of chloroplast functional capacity, often decreasing with starvation of the sacoglossan (GREENE, 1970b; CLARKE & BUSACCA, 1978).

In the summers of 1983 and 1984, three salt marshes in the Minas Basin, Nova Scotia, contained populations of *Elysia chlorotica* Gould, 1870 (Opisthobranchia: Sacoglossa) ranging from light green to non-green in color, instead of the usual rich dark green that results from the presence of endosymbiotic chloroplasts derived from the food alga *Vaucheria* sp. (BAILEY & BLEAKNEY, 1967; GRAVES *et al.*, 1979). A survey of the literature indicated that a naturally occurring population of symbiotic elysiid sacoglossans not strongly pigmented by chloroplasts had never been reported.

The aim of this study was to use the above population as the basis for an examination of sacoglossan color and chloroplast function as related to chlorophyll content, oxygen production, and oxygen consumption.

MATERIALS AND METHODS

Collection and Maintenance of Animals

Elysia chlorotica individuals were collected from salt marshes located at Kingsport, Pickett's Wharf, and Porter's Point in the Minas Basin. They were usually found on exposed mats of an undetermined *Vaucheria* species along the edges of pools and creeks, on pool bottom sediments, or on an assortment of submersed algae, including *Rhizoclonium, Cladophora,* and *Ectocarpus* species.

The slugs were divided into three study groups by comparison with MUNSELL (1977) color charts. Color groupings used were as follows: Group 1 (dark green) ranged in color from 7.5GY 4/4, 4/6 to 5GY 7/6, 7/8, Group 2 (light green) from 5GY 7/6, 7/8 to 2.5GY 7/4, 7/6, and Group 3 (non-green) from 2.5GY 7/4, 7/6 to 5Y 8/4, 8/6. All specimens were used as soon as possible after

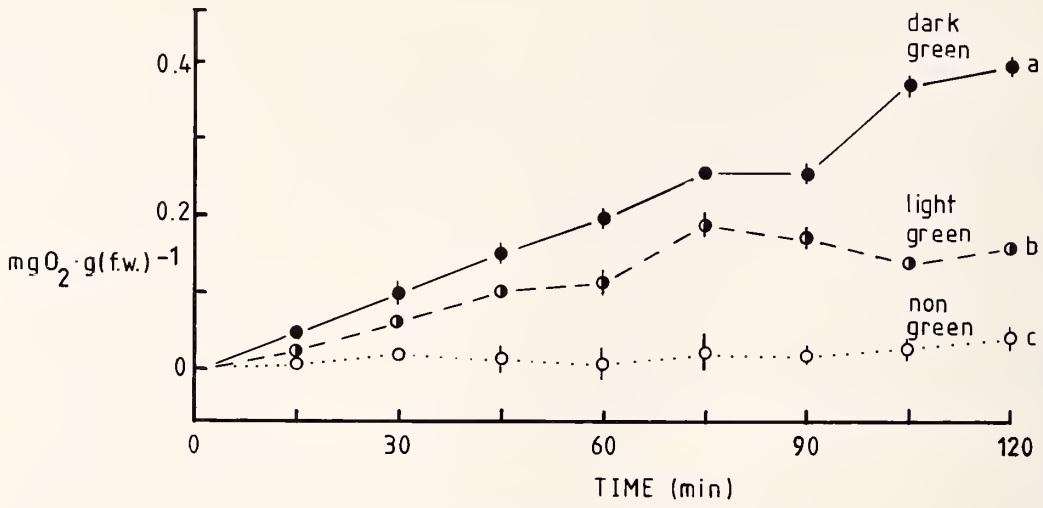

Figure 1

Gross O_2 production of each *Elysia chlorotica* study group. Mean values are plotted, and SE bars included where the standard error is greater than the area covered by the mean symbol. n = 6 for each group. a, Group 1; b, Group 2; c, Group 3.

collection and were maintained in the dark at 9°C and at 28‰ salinity without food between tests.

Measurements of Oxygen Production and Consumption

Photosynthetic and respiratory activity were compared between study groups by measuring light-dark O_2 production and consumption. Control tests without slugs were also conducted. Slugs were gently blotted to remove excess water, weighed, and placed in a stoppered 12-mL flask filled with O_2-saturated (PO_2 = 155 mm Hg) seawater (28‰ salinity). Changes in oxygen pressure were measured at 15-min intervals for 2 h with a Radiometer O_2-electrode system. Illumination was provided by a 120-Volt American Optical fluorescence lamp with two bulbs positioned at right angles to each other and at a 1-cm distance from the flask. During the dark trials, the flask was wrapped in aluminum foil and continuous illumination was maintained to eliminate the possibility of light-induced temperature changes (1.2–2.0°C) affecting the electrode characteristics and O_2 solubility. The water was mixed with a small magnetic stirrer and the electrode allowed to stabilize before each reading was taken. PO_2 was converted to mg O_2 with the following equation (adapted from Hoar & Hickman, 1975):

$$\text{mg } O_2 \cdot g(fw)^{-1}\ h^{-1} = \frac{PO_2 \text{ mm Hg} \cdot 1000\alpha \cdot 1.43}{BP \cdot g(fw) \cdot h}$$

where g(fw) = fresh weight of the slug in grams, h = time (in hours), α = the appropriate O_2 solubility coefficient alpha for a specific temperature, 1.43 = a conversion factor to change mL O_2 to mg O_2, and BP = barometric pressure. Gross O_2 production was determined by adding the amount of O_2 consumed in the dark to that produced in the light. Rates of respiration (O_2 consumed in the dark) were compared using a Mann-Whitney U test.

Measurement of Chlorophyll Content

Animals were gently blotted to remove excess water, weighed, anaesthetized at −9°C for 2–3 min, and homogenized in 2 mL of absolute methanol. The suspension was centrifuged in a IECHT centrifuge at 8000 rpm for 10 min. The centrifuged pellet was washed twice with absolute methanol and the extracts combined for a total volume of 5 mL. Samples were stored temporarily (less than 1 h) in the dark at 9°C to prevent bleaching. Chlorophyll content (chlorophylls *a* and *c* inclusive) was determined with a Varian Techron model 635 spectrophotometer using the following equation (after MacLachlan & Zahlik, 1963):

$$\text{mg chl } g(fw)^{-1} = 25.5(A_{650}) + 4.0(A_{655}) \cdot V/(g(fw) \cdot 1000)$$

where g(fw) = fresh weight of slug in grams, A = absorption at the indicated wavelength, and V = total volume (5 mL) of methanol extract.

OBSERVATIONS

The three study groups showed some gross O_2 production (Figure 1). Group 1 (dark green slugs) have both the greatest O_2 production and the highest chlorophyll content (Table 1), Group 2 (light green slugs) showed less O_2 production and a lower chlorophyll content, while Group 3 (non-green slugs) showed almost no O_2 production and a very low chlorophyll content. Groups 2 and 3 consumed O_2 at a greater rate than O_2 was produced, in contrast to

Table 1

Chlorophyll content, O_2 production, and O_2 consumption for each *Elysia chlorotica* study group. Values given are means ± SE. n = 6 for each group.

Group	Group 1 (dark green)	Group 2 (light green)	Group 3 (non-green)
Chlorophyll content			
mg chl g $(fw)^{-1}$	1.83 ± 0.16	0.59 ± 0.05	0.16 ± 0.01
mg O_2 $g(fw)^{-1}$ h^{-1}			
O_2 produced in light	0.0775 ± 0.0095	−0.0193 ± 0.0073	−0.0828 ± 0.0097
O_2 consumed in dark	−0.1109 ± 0.0029	−0.1060 ± 0.0082	−0.0913 ± 0.0038
Gross O_2 production	0.1884 ± 0.0119	0.0883 ± 0.0125	0.0122 ± 0.0125
mg O_2 $g(fw)^{-1}$ g chl^{-1} h^{-1}	0.1029	0.1500	0.0762

Group 1. O_2 produced per unit chlorophyll was greatest in Group 2, and lowest in Group 3. The three study groups displayed similar rates of respiration (O_2 consumption in the dark).

DISCUSSION

Although a naturally occurring non-green elysiid population has not previously been reported, the relationship between chlorophyll content, an indicator of chloroplast functional capacity (Greene, 1970b), and sacoglossan starvation has been studied by several authors (Trench *et al.*, 1969; Clark *et al.*, 1981). Results vary with species. Chlorophyll levels have been found to decrease after a 24-h starvation period in *Elysia hedgpethi,* accompanied by a parallel decrease in chloroplast functional capacity (Greene, 1970b). In the same study, Greene observed that chlorophyll levels in *Placobranchus ianthobapsus* remained unaffected throughout a 27-day starvation period, while the photosynthetic ability of the chloroplasts decreased. In *Elysia viridis,* chlorophyll levels increased over a 93-day starvation period and chloroplasts remained functional for 3 months (Hinde & Smith, 1972). Clark & Busacca (1978) found that chlorophyll content decreased with starvation in four sacoglossan species: *Elysia tuca, Tridachia crispata, Oxynoe antillarum,* and *Elysia cauze.*

It could not be determined whether or not starvation had occurred before collection of the naturally pale *Elysia chlorotica* specimens examined in this study, and if it did, for what length of time. However, there is a relationship between chlorophyll content and chloroplast functional capacity. *Elysia chlorotica,* from all three study groups, showed some gross O_2 production. Group 1 (dark green slugs) had both the greatest chlorophyll content and the largest O_2 production. Although less O_2 was produced by the animals in Group 2 (light green slugs), each unit of chlorophyll produced 31% more O_2 than in Group 1. Animals in Group 3 (non-green slugs) showed almost no gross O_2 production, the chlorophyll content decreased to 9% of that of Group 1, and each unit of chlorophyll produced 26% less O_2 than in Group 1. The increased O_2 production per unit of chlorophyll in Group 2 is of interest, although difficult to explain. Possibly as chlorophyll is lost, more light is able to penetrate the sacoglossan tissue, or some means of regulating chlorophyll activity occurs. However, it appears that *E. chlorotica* can maintain a high level of O_2 production while chlorophyll levels are starting to decline. As chlorophyll levels continue to fall, this ability is lost, as shown in Group 3.

All three study groups had equivalent rates of respiration (Groups 1 and 3 compared: Mann-Whitney U = 48.5, $P > 0.05$, $n_1 = 9$, $n_2 = 9$). This is indicative of the tissues being in a similar physiological condition, regardless of color. Variations observed might have been influenced by several factors. Specimens used were selected from a certain size range (4–12 mm) but the variation within this range would result in differing rates of respiration (Sander & Moore, 1978). Respiration rates may also be affected by the small temperature changes that occurred (Sander & Moore, 1978) as well as by the change in O_2 tension in the experimental chamber as O_2 was consumed during each test (Mandan Mohan Das & Venkatachari, 1984).

The cause for the appearance of the naturally pale *Elysia chlorotica* population is not known. Field records for the salt marshes of the Minas Basin (Bleakney, 1966–1982, unpublished data), report that only two light green *E. chlorotica* individuals have been previously collected (May 1, 1969). Field records for 1983 indicate that almost all of the algae in and around the marsh pools were dead by late May, probably as a result of heavy rainfall throughout the month. In 1984, *Vaucheria* mats did not appear until the end of June. Perhaps the loss of green pigment was related to the inability of the slugs to locate sufficient *Vaucheria.* This would either result in starvation or force the slugs to find another food source, presumably without compatible chloroplasts. However, starvation of this species does not usually produce a loss of green pigment. Dark green *E. chlorotica* collected in other years have remained healthy and green for at least 4 months in a 9°C refrigerator. S. K. Pierce (1984, *in litt.*) was also unable to bleach out the chlorophyll through starvation of this species. Dark green slugs collected in the Minas Basin

in the summers of 1983 and 1984 did lose chlorophyll when starved for 2 to 3 wk. Non-green slugs fed freshly collected *Vaucheria* sp. turned green within 4 days. Perhaps the *Vaucheria* ingested during this period was in some way debilitated, causing the chloroplasts to bleach more rapidly.

Examination of a naturally occurring green and non-green *Elysia chlorotica* population indicates that there is a relationship between declining chlorophyll content (reflected in slug color) and the photosynthetic ability of the endosymbiotic chloroplasts. The functional capacity of the total amount of chlorophyll present seems to vary as pigment is lost. Regardless of color, the rate of O_2 consumption does not appear to change.

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